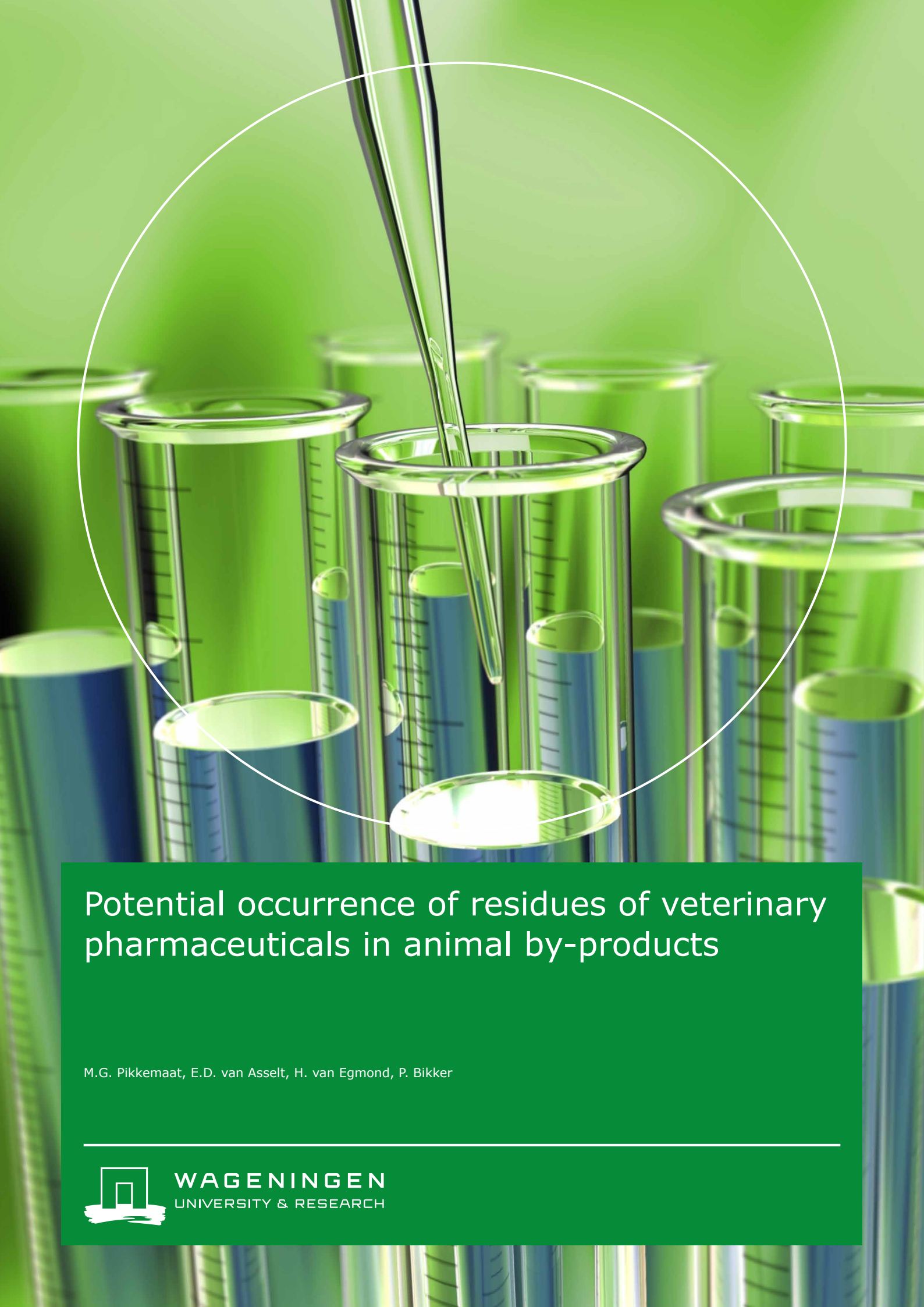

Potential occurrence of residues of veterinary pharmaceuticals in animal by-products

M.G. Pikkemaat, E.D. van Asselt, H. van Egmond, P. Bikker

WAGENINGEN
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Contents

Summary

Animal by-products may be used as ingredient in animal feed under certain conditions. Examples are fish meal or blood plasma. These by-products may originate from countries outside Europe in which legislation related to the use of veterinary drugs may be different from the EU. Consequently, other pharmaceutically active substances (PAS) may be allowed for use in food producing animals. A previous inventory identified a total of 104 substances for which maximum residue limits (MRLs) are specified in non-EU countries, while these are not authorized for use in food producing animals within the EU. The aim of the current study was to gain more insight into the potential use of these substances in a selection of non-EU countries by determining to what extent veterinary pharmaceutical products (VPPs) based on these substances are available.

The study comprised Brazil, China and the United States (USA), which were selected for further research becaAe of their significant export volumes, while India and Indonesia were included because of the large number of substances of interest. For each of these countries, VPP databases were searched for available products and target animal species were retrieved as far as possible. The current research provided better insight in the availability of VPPs for the PAS of interest. For Brazil, China and USA, substantial numbers of products were found for the majority of the PAS, indicating towards actual use. For India, at least half of the PAS of interest was not represented by-products in the VPP data and for the remaining substances the distinction between animal and human medicine was often unclear. Overall the Indian resources contained an unrealistically low number of VPPs. For Indonesia VPPs were found only for 6 PAS, probably indicating obsolete MRL legislation.

It was remarkable to note that, in particular for Brazil and Indonesia, the VPP databases contained a substantial number of products based on PAS lacking an EU-MRL, which were not identified in the initial study. This suggests considerable gaps in prevailing legislation and should be scrutinized in future Health and food audits and analysis (HFAA) audits.

The results of this study provide a more realistic view on potential or (un)likely use of PAS, and consequently on potentially relevant residues. It allows for a more specific targeting of the monitoring of animal (by-)products, depending on the country of origin and the animal species. It should be noted, however, that the actual risk of residues occurring in animal by-products also depends on the pharmacokinetics of the PAS, which was not taken into account in this study. Finally, it is recommended to define tolerance levels for PAS in animal by-products to facilitate enforcement.

1 Introduction

Animal by-products, such as fish meal or blood plasma, may be used as ingredients in animal feed. When animals are treated with or exposed to veterinary pharmaceutical products (VPPs), residues may end up in the animal by-products. Regulation (EU) 37/2010 comprises the pharmacologically active substances (PAS) that are authorised for use in food producing animals within the EU and provides maximum residue limits (MRLs) for animal products. An additional limited number of PAS (in particular coccidiostat feed additives) have MRLs defined under their respective implementing regulations. Countries outside the EU, however have their own regulations and approval procedures. As a consequence, animal by-products originating from these countries may contain residues of veterinary drugs that are not authorised within the EU. In 2021, an inventory was performed comprising 20 non-EU countries that are most relevant with respect to import of animal by-products in the Netherlands (van Asselt et al., 2021). This inventory revealed that a total of 104 pharmaceutically active substances, for which an MRL was defined in (one or more) of these countries, but for which no EU-MRL exists.

The presence of an MRL does not necessarily imply that products containing these active substances are actually available on the market and used in animal production. Maximum residue limits can for example concern obsolete substances that were used in the past and have been superseded by more appropriate active substances. Maximum residue limits may also have been established to facilitate international trade. An example is cyromazine, which has never been applied for veterinary purposes in the EU. Nevertheless, an EU-MRL in sheep was established in order to facilitate the import of sheep products from Australia and New Zealand. The substance is used in this country for prevention and treatment of ectoparasites. In the EU, cyromazine is only approved as a pesticide.

In most countries, formal registration of a veterinary pharmaceutical product is a prerequisite before entering the market. The availability of registered products, in particular for use in food producing animals, therefore, is considered a more reliable indicator for potential use.

Aim of the project
The aim of this project was to determine to what extent veterinary pharmaceutical products (VPPs) based on substances that lack authorisation for use in food producing animals in the EU, are available in countries outside the EU.

Following the initial study, five countries were selected for further research. Brazil, China and the United States of America (USA) were selected as the main countries exporting animal by-products to the Netherlands. For these countries, 10, 28 and 27 PAS of interest were identified, respectively in the previous study (van Asselt et al., 2021). Furthermore, India and Indonesia were selected, as these have a relatively large number of PAS not authorised in the EU (28 and 44 substances, respectively). For each of the countries, VPP databases were searched for available products. If available, the target animal species were identified for these products.

2 Material and methods

For each of the five countries, i.e. China, Brazil, USA, India and Indonesia, a search strategy for VPP registrations was developed and applied. Localization of relevant information regarding authorised veterinary products that contained PAS unauthorised within the EU, was pursued via Google Search, our WFSR network of experts and via contacting the Agricultural Boards of the five countries. Once relevant databases and/or product overviews were identified, these were screened for products containing the relevant PAS. Table 1 provides an overview of the investigated substances for each of the individual countries.

Table 1 *Pharmaceutically active substances authorised for food producing species in Brazil, China, the USA, India and Indonesia but lacking EU authorisation.*

Substance	Category[1]	Brazil	China	United States	India	Indonesia
1,2-Dichlorobenzene	A3b	-	-	-	x	-
Acepromazine	A3f	-	-	-	x	-
Antipyrine	B1d	-	-	-	x	-
Arsanilic acid	A3d	-	x	-	-	-
Avoparcin	A3c	-	-	-	-	x
Berberine	-	-	x	-	-	-
Buparvaquone	A3d	-	-	-	x	-
Buquinolate	A3d	-	-	-	-	x
Cambendazole	A3b	-	-	-	-	x
Carbadox	A3c	-	-	x	-	x
Carbofuran	A3b	-	-	-	-	x
Carboprost tromethamine	-	-	-	-	x	-
Chloral hydrate	A3f	-	-	-	x	-
Chloramphenicol	A2	-	-	-	-	x
Chlordimeform	A3b	-	-	-	-	x
Chlorpromazine	A2	-	x	-	-	-
Chlorpyridazine	-	-	-	-	x	-
Clindamycin	A3c	-	-	-	-	x
Clopidol	A3d	-	x	x	-	x
Coumaphos	B1b	x	-	x	-	x
Cymiazole	A3b	-	-	-	-	x
Dapsone	A2	-	-	-	-	x
Destomycin A	A3c	-	x	-	-	-
Dexcloprostenolum	-	-	-	-	x	-
Diazepam	A3f	-	x	-	-	-
Dichlorvos	A3b	-	x	x	-	x
Diethylcarbamazine	A3b	-	-	-	x	-
Dimetridazole	A2	-	x	-	-	x
Diminazine	A3b	x	x	-	-	-
Dinitolmide (Zoalene)	A3d	-	x	x	x	-
Efrotomycin	A3c	-	-	x	-	-
Estradiol	A1c	x	x	x	-	-
Ethopabate	A3d	-	x	x	x	x
Famphur	A3b	-	-	x	-	x
Fenprostalene	-	-	-	x	-	-
Fenthion	A3b	-	x	x	-	x
Flavomycin[#]	(A3c)	-	-	x	x	-
Fluvalinate	A3b	-	x	-	-	x
Furaltadone	A2	-	-	-	-	x
Furazolidone	A2	-	-	-	-	x
Gonadotropin	A3e	-	x	x	x	-

Substance	Category[1]	Brazil	China	United States	India	Indonesia
Haloxon	A3b	-	-	x	x	x
Halquinol	A3c	x	-	-	-	-
Hygromycin B	A3c	-	x	-	-	x
Isometamidium	A3b	x	x	-	-	x
Kaolin	-	-	x	-	x	-
Kitasamycin	A3c	-	x	-	-	x
Laidlomycin	A3d	-	-	x	-	-
Lindane	A3b	-	-	-	-	x
Lithium antimony thiomalate	A3f	-	-	-	x	-
Lubabegron	A1e	-	-	x	-	-
Malathion	A3b	-	x	-	-	-
Melengestrol acetate	A1c	x	-	x	-	x
Mepyramine	-	-	-	-	x	-
Methyl hydroxybenzoate	-	-	-	-	x	-
Metoserpate hydrochloride	A3f	-	-	x	-	x
Metronidazole	A2	-	x	-	-	-
Nandrolone	A1c	-	x	-	x	-
Naphthalophos	A3b	-	-	-	-	x
Nequinate	A3d	-	-	x	-	x
Niclosamide	A3b	-	-	-	x	-
Nifurstyrenate	A2	-	-	-	-	x
Nimesulide	A3f	-	-	-	x	-
Nitrofurazone	A2	-	-	-	-	x
Nitroscanate	A3b	-	-	-	x	-
Norfloxacin	A3c	-	-	-	-	x
Olaquindox	A3c	-	x	-	-	x
Oleandomycin	A3c	-	-	-	-	x
Ormetoprim	A3d	-	-	x	-	x
Pentobarbitone	A3f	-	-	-	x	-
Polymyxin B	A3c	-	-	-	-	x
Proligestone	A1c	-	-	-	x	-
Promazine hydrochloride	A3f	-	-	-	x	-
Propetamphos	A3b	-	x	-	-	-
Propofol	A3f	-	-	-	x	-
Pyrantel	B1b	-	-	x	-	x
Quinapyramine	A3b	-	-	-	x	-
Ractopamine	A1e	x	-	x	-	-
Ronidazole	A2	-	-	-	-	x
Roxarsone	A3d	-	x	-	-	-
Scopolamine	-	-	x	-	-	-
Sulfomyxin	A3c	-	-	-	-	x
Suramin	A3b	-	-	-	x	-
Temephos	A3b	-	-	-	-	x
Testosterone	A1c	x	x	x	-	x
Trenbolone	A1c	-	-	x	-	-
Tribromsalan	A3c	-	-	-	-	x
Trichlorfon	A3b	x	x	-	-	x
Tripelennamine	-	-	-	x	-	-
Zeranol	A1d	-	-	x	-	x
Zilpaterol	A1e	x	-	x	-	-
Total		10	28	27	28	44

[1] Category according to Implementing Regulation (SANTE 10216-2022).

[#] Flavomycin (bambermycin) was banned as antimicrobial growth promoter in the EU, but recently reintroduced as veterinary drug for rabbits (no MRL necessary) and poultry (MRLs apply).

3 Results

For each of the five investigated countries, databases or lists of approved veterinary pharmaceutical products were found and evaluated for the presence of products based on substances that are unauthorised for use in food producing animal species in the EU. The results are indicated in the sections below.

3.1 Brazil

The national competent authority responsible for the evaluation and registration of VPPs in Brazil is the Ministry of Agriculture, Livestock and Supply (MAPA). Brazil's veterinary drug registrations can be accessed through a comprehensive electronic dashboard available from: https://www.gov.br/agricultura. This database was screened for the presence of VPPs based on the substances previously identified and displayed in Table 1. This yielded the result shown in Figure 1.

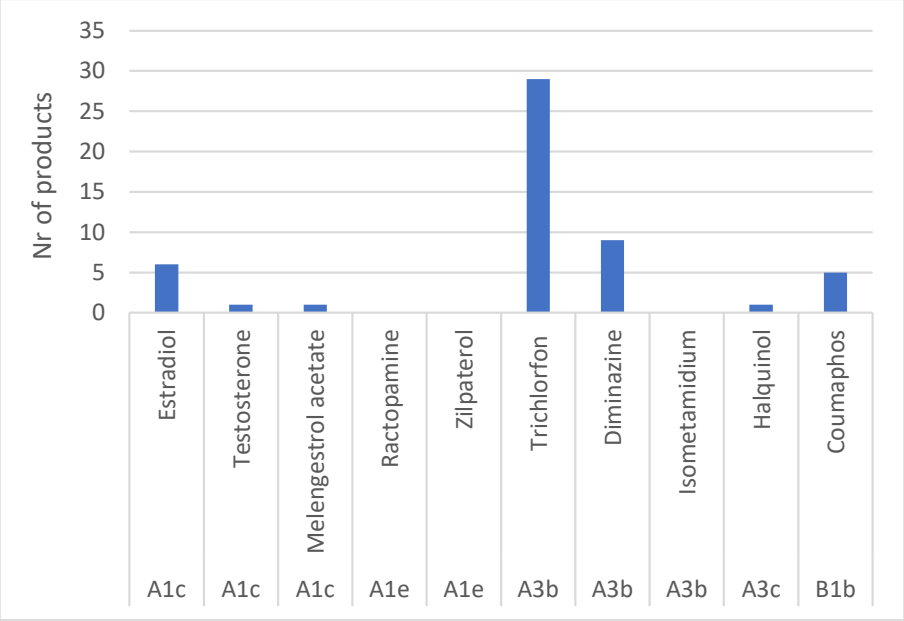

Figure 1 *Number of products found in Brazil containing substances that are not authorised in the EU.*

For some of the PAS (ractopamine, isometamidium and zilpaterol) no VPPs were found in the database. For most of the other substances, the number of products was limited, except for trichlorfon (a.k.a. metrifonate) for which 29 registered products were available. This organophosphate insecticide is used to control ectoparasites like ticks and lice in a wide range of target species including ruminants, horse, pigs, poultry and fish. Diminazene was found in 9 products. This is an anti-infective for treatment of protozoal diseases. Target species of the authorised products are primarily ruminants and horse, and to a lesser extent also pig (Table 2). Target species for the hormonal substances are primarily bovines. Halquinol appears to be applied mainly as a feed additive for poultry and pig. Coumaphos is not an unauthorised substance in the strict sense within the EU, as it has an MRL in honey, but applications for other food producing species are unauthorised. In Brazil, however, VPPs containing this substance have been approved for several mammalian species.

Table 2 *Target animal species associated with VPPs available in Brazil.*

Substance	Target animal species
A1c - Estradiol	bovine, horse, dog, goat, cat, swine, ovine
A1c - Testosterone	bovine
A1c - Melengestrol acetate	bovine
A3b - Trichlorfon	bovine, horse, ovine, swine, goat, poultry, dog
A3b – Diminazine	bovine, horse, goat, sheep, swine, dog
A3b - Isometamidium	bovine
A3c – Halquinol	poultry, swine, bovine
B1b - Coumaphos	bovine, horse, dog, goat, sheep, swine

It is worth noting that the MAPA veterinary products database revealed a considerable number of VPPs based on substances not identified in the 2021 inventory study. An overview of these PAS is given in Table 3.

Table 3 *Additional substances that are not authorised for food producing species in the EU, for which authorised VPPs were found in Brazil.*

	Substance	Target animal species	Remark
A3b	Azamethiphos	Bovine	EU: no MRL required fin fish
A1c	Boldenon	Horse only	
-	Carbaryl	Poultry/bovine/sheep/goat/swine	
A3b	Chlorfenvinphos	Bovine/goat/sheep	
A3b	Chlorpyrifos	Bovine/sheep/poultry/swine	
A3d	Diaveridine	Poultry/rabbits/swine/sheep	
A3b	Dichlorvos	Bovine/goat/sheep/horse	
A3b	Dichlofenthion	Bovine	Only one product
A3b	Disophenol	Bovine/ovine/goat/swine	
A3c	Enramycin	Poultry/swine	
-	Ergomethrin	Bovine/horse	
A3b	Ethion	Bovine	
A3b	Fenitrothion	Bovine/sheep/goat/swine	
A3b	Fenthion	Bovine/sheep/goat	
A3f	Phenylbutazone	Horse/bovine/swine	According to legislation not allowed*
A3b	Fipronil	Bovine/sheep/goat	
A3c	Fosfomycin	Poultry/swine	
A3a	Gentian violet	Bovine/goat/sheep/swine	According to legislation not allowed*
A3c	Leucomycin	Swine	
A3c	Miconazol	Bovine	
A3c	Norfloxacin	Bovine/poultry/swine/ovine	
-	Propoxur	Poultry/bovine/goat/sheep/porcine	
A3b	Tetrametrin	Bovine/ovine/swine/poultry	

* INSTRUÇÃO NORMATIVA N° 51, DE 19 DE DEZEMBRO DE 2019 Estabelece a lista de limites máximos de resíduos (LMR), ingestão diária aceitável (IDA) e dose de referência aguda (DRfA) para insumos farmacêuticos ativos (IFA) de medicamentos veterinários em alimentos de origem animal.

A considerable number of substances in this list are organophosphates or pyrethroids (and even carbamates, a chemical class of pesticides banned for animal use in the EU) primarily used for prevention or treatment of ectoparasites. Enramycin is a feed additive that is not well absorbed and is assumed not to leave significant residues in edible tissues. Fosfomycin and norfloxacin are examples of antibiotics not approved for food producing animal species in the EU. The approval of fipronil for use in ruminant species is most remarkable (Table 3). In Europe, this substance is associated with (illegal) application in poultry.

It is unclear how to explain the fact that products based on substances in Table 3 seem to be authorised for use in livestock species, while no MRLs are specified for these substances in the applicable Brazilian legislation (Instrução Normativa nº 51). Apparently, during the most recent audit of the Health and Food Audits and Analysis (HFAA), the digital database was not available yet. At that time, the evaluation of

available veterinary products was based on a "DFIP internal list of authorised pharmacologically active substances" (European Commission, 2018a). Since the MAPA dashboard has gone live only recently, it might be possible that the database is not up to date yet.

3.2 China

The national competent authority responsible for the evaluation and registration of VPPs in China is the Chinese Institute of Veterinary Drug Control (IVDC)/Centre for Veterinary Drug Evaluation (CVDE) of the Ministry of Agriculture and Rural Affairs (MARA). Veterinary Pharmaceutical Product registrations are available from the National Veterinary Drug Basic Information Database which can be accessed through: http://124.126.15.169:8081/cx/.

The database contains currently approved as well as expired and withdrawn products and was screened for the presence of VPPs based on the PAS indicated in Table 1. The number of currently approved VPPs containing these substances is indicated in Figure 2. Target animal species can be found in Table 4.

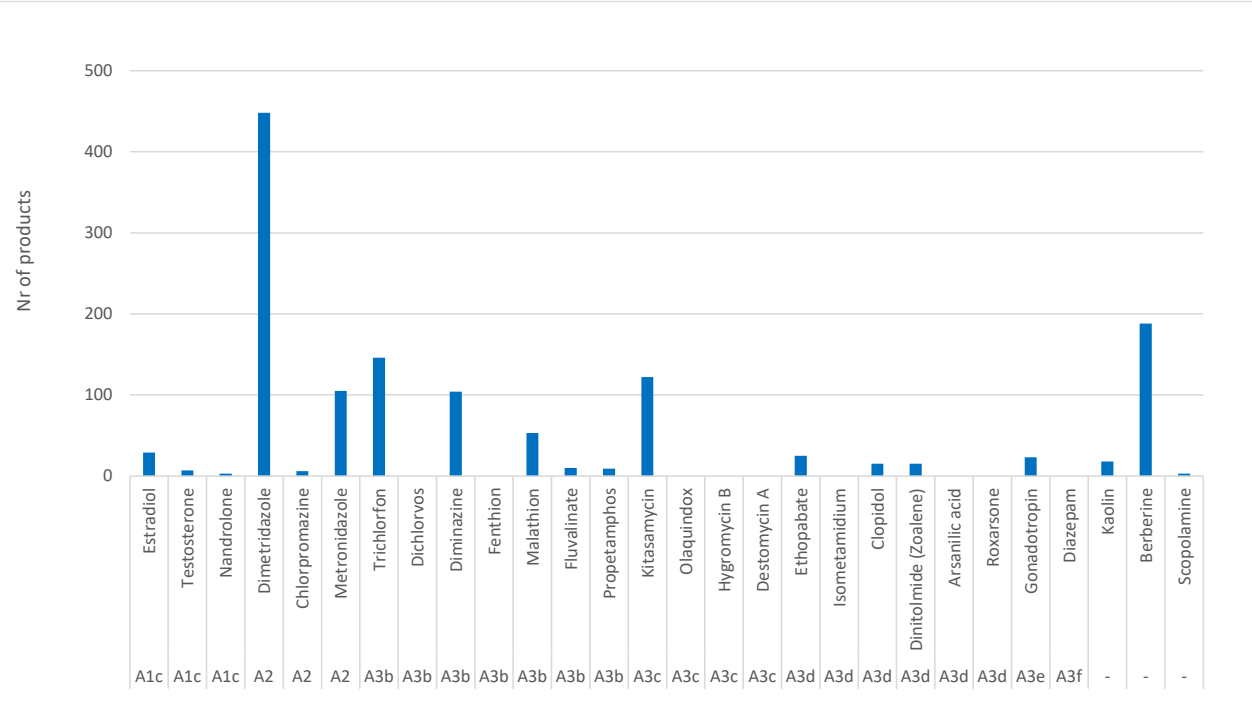

Figure 2 *Number of currently approved products found in China containing substances that are not authorised in the EU.*

For a limited number of substances, the database did not show any (currently approved) products: dichlorvos, fenthion, isometamidium, olaquindox, hygromycin, arsanilic acid, destomycin, roxarsone and diazepam. Some of these are former feed additives. Olaquindox, arsanilic acid and roxarsone permits were withdrawn in 2018 under Announcement No. 2638 of the Ministry of Agriculture (http://www.moa.gov.cn). Hygromycin and destomycin products were approved as feed additive and included in the medical feed additive catalogue (MOA Notice No. 168) until a few years ago. However, in 2019 MARA published Announcement No. 194, terminating the legal use of all growth-promoting drug feed additives (except traditional Chinese medicine) by January 2020, and revoking announcement No. 168 and the supplementing announcement No. 220.

Figure 2 shows that there is a large number of products authorised containing dimetridazole (n = 448) or metronidazole (n = 105). These nitroimidazoles are prohibited for use in food producing species in the EU and a minimum method performance requirement (MMPRs) of 1 ppb in products of animal origin applies to

these substances (EURL, 2022). For the other A2 substance, chlorpromazine, 6 products were found in the database. However, these were registrations for companion animals only. Substantial numbers of VPPs were found for the antiparasitics trichlorfon (n= 146) and diminazine (n= 104). Notably, while in Brazil trichlorfon is exclusively registered for mammalian species, in China the vast majority of trichlorfon products is indicated for use in aquaculture. Other frequently found substances were kitasamycin (n = 122), an antibacterial growth promotor that apparently was not banned after MOA Announcement No. 194 and berberin (n= 188), which, like scopolamine, is an alkaloid used in traditional Chinese medicine.

Table 4 *Target animal species associated with currently approved VPPs available in China.*

	Substance	**Target animal species**
A1c	Estradiol	Cattle, sheep/goat, pig
A1c	Testosterone	Livestock
A1c	Nandrolone	Livestock
A2	Dimetridazole	Pigs, chickens
A2	Chlorpromazine	Dogs, cats
A2	Metronidazole	Dog, cat, cattle
A3b	Trichlorfon	Fresh water aquaculture fish, horse, cattle, sheep, goat, pig
A3b	Diminazene	Horse, cattle, sheep
A3b	Malathion	Livestock (highly toxic to fish)
A3b	Propetamphos	Sheep (toxic to poultry and fish)
A3c	Kitasamycin	Swine, poultry
A3d	Ethopabate	Chicken
A3d	Clopidol	Chicken, rabbit
A3d	Dinitolmide	Poultry
A3e	Gonadotropin	Horse, cow, sheep/goat, pig, dog, cat, rabbit, mink
-	Kaolin	Horse, cattle, sheep/goat, pig, dog
-	Berberine	Horse, cattle, sheep, pig
-	Scopolamine	Cattle, sheep/goat, pig

The Chinese National Veterinary Drug Basic Information Database was also screened for potential presence of VPPs based on additional substances for which MRLs were found in the other 4 countries (see Table 1). To achieve this, these substances were translated into Chinese using google translate and then submitted to the database. This yielded three additional substances of interest. Several premixes for poultry, cattle and pig based on flavomycin were found. Flavomycin (also known as flavophospholipol or bambermycin) was banned as an antimicrobial growth promoter in the EU, but was recently reintroduced as veterinary drug for rabbits (no MRL necessary) and poultry (MRLs apply). However, the Chinese database refers to an announcement No. 246, which might imply that it is no longer allowed (as a feed additive). A substantial (n=82) number of records concerning niclosamide was found, for multiple food producing target species (cattle, sheep, pig, aquaculture). This PAS is primarily used to treat tapeworms and in the EU it is only registered for companion animals. The largest number of records was found for ciprofloxacin (>1000). This fluoroquinolone antibacterial is officially not allowed in the EU for use in food producing animal species. It is covered by standard residue monitoring methods, since the MRL of enrofloxacin is defined as the sum of enrofloxacin and its metabolite ciprofloxacin.

3.3 USA

The national competent authority responsible for the evaluation and registration of VPPs in the USA is the Food and Drug Administration (FDA). Drugs and medicated feeds are regulated under the Code of Federal Regulations (CFR) Title 21 "Food and Drugs" (https://www.ecfr.gov/current/title-21) Subchapter E.

Registered products were retrieved from the FDA website Animal Drugs @ FDA (https://animaldrugsatfda.fda.gov/adafda/views/). The Green Book report "Section 2: Active ingredients" comprises a list of registered products based on application number, indicating the active ingredient and trade name of the product. This yielded the results shown in Figure 3.

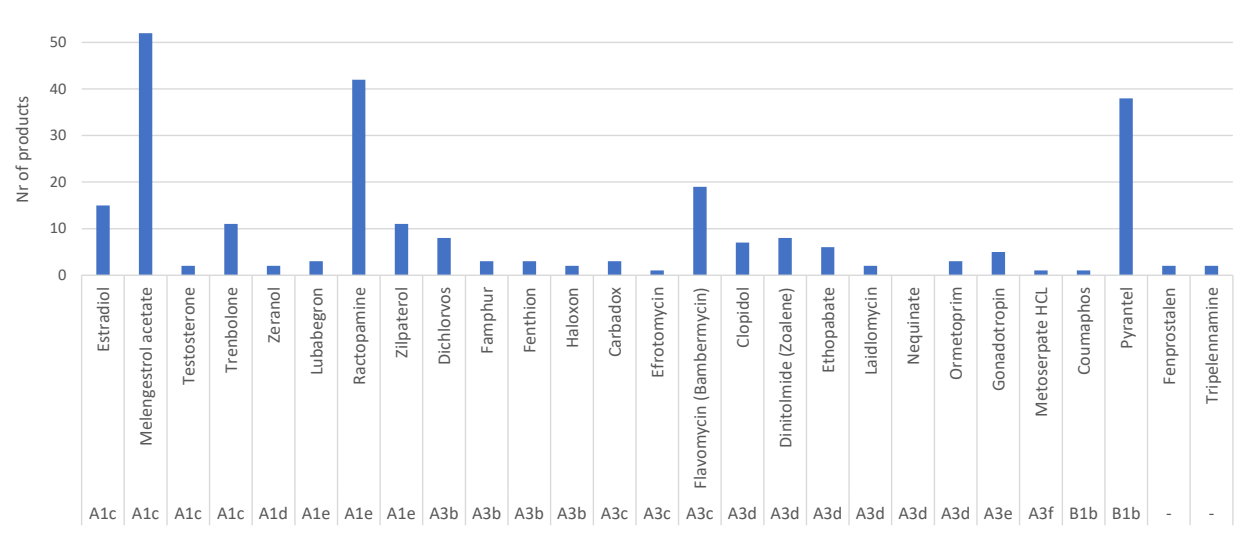

Figure 3 *Number of currently approved / authorised products found in the USA containing substances that are not authorised in the EU.*

Products were found for all but one (nequinate) substances previously identified and indicated in Table 1.

The overview of products provided by the Green Book report section 2 does not provide details with respect to target species, therefore this was derived indirectly from CFR Title 21 part 556 (Tolerances for Residues of New Animal Drugs in Food) and part 558 (New Animal Drugs for Use in Animal Feeds). An overview can be found in Table 5. This table shows that about half of the PAS of interest are applied in animal feed. Many of the substances in this table are used as growth promoters, reflecting the different attitude towards the use of growth promoting substances in the USA as compared to the EU.

Table 5 *Target animal species associated with VPPs available in the USA.*

	Substance	Target animal species	Feed application
A1c	Estradiol	cattle	-
A1c	Melengestrol acetate	cattle	-
A1c	Testosterone	cattle	-
A1c	Trenbolone	cattle	-
A1d	Zeranol	cattle, sheep	-
A1e	Lubabegron	cattle	Y
A1e	Ractopamine	cattle, swine, turkey	Y
A1e	Zilpaterol	cattle	Y
A3b	Dichlorvos	swine	Y
A3b	Famphur	cattle	Y
A3b	Fenthion	cattle	-
A3b	Haloxon	cattle	-
A3c	Carbadox	swine	Y
A3c	Efrotomycin	swine	Y
A3c	Flavomycin (Bambermycin)[#]	cattle, swine, poultry	Y
A3d	Clopidol	poultry	Y
A3d	Dinitolmide (Zoalene)	poultry	Y
A3d	Ethopabate	poultry	-
A3d	Laidlomycin	cattle	Y
A3d	Ormetoprim	poultry, salmonids, catfish	Y
A3e	Gonadotropin	swine, finfish	-
A3f	Metoserpate HCL	chicken	-
B1b	Coumaphos	chicken (layer)	Y
B1b	Pyrantel	swine, horse	Y
-	Fenprostalene	cattle, swine	-
-	Tripelennamine	horse, cattle	-

[#] Flavomycin (bambermycin) was banned as antimicrobial growth promoter in the EU, but recently reintroduced as veterinary drug for rabbits (no MRL necessary) and poultry (MRLs apply).

Besides growth promoting substances, the list contains several antiparasitics, like dichlorvos, famphur and fenthion (insecticides), haloxon (anthelmintic) and ethopabate (coccidiostat). Ormetoprim is a trimethoprim analogue and is used in combination with sulfonamides, similar to trimethoprim. Metoserpate is a tranquilizer used for stress reduction in poultry. Fenprostalen is a potent prostaglandin analogue used for oestrus synchronization, but also to induce parturition or abortion. Tripelennamine is an antihistamine used to treat allergies. As mentioned earlier, coumaphos is approved for bees in the EU. In the USA, however, application for poultry is approved. A similar situation holds true for pyrantel, which is approved for horses (no MRL necessary) but is also applied in pigs in the USA.

The USA regulations were also screened for potential presence of VPPs based on additional substances. For two of the substances represented in Table 1, VPPs for food producing species were found: sulfomyxin for use in poultry and hygromycin for use in chicken and swine. For both substances, tolerance levels, however, were set at zero, explaining why these substances were not identified using our earlier search strategy in USA context. Additionally, the in-feed application of thiabendazole as an anthelmintic for use in swine, cattle, sheep, goats and pheasants was found. USA tolerance levels for this substance in edible tissue and milk are 0.1 and 0.05 ppm, respectively. It is unclear why this substance was not identified in the earlier study.

3.4 India

The national competent authority in India, the Central Drugs Standard Control Organization (CDSCO) (under the Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India) hosts a website on which three lists (comprising different time periods) covering veterinary drugs approved by CDSCO can be found: Approval New https://cdsco.gov.in/. The information, however, is provided with the disclaimer that it is only to be used for reference purposes and not for statutory clearance. The initial chronological list provided by CDSCO ("List of Veterinary Drugs") comprises registrations dating back to 1969. It is unclear whether registrations are periodically reviewed or removed from the list when products become obsolete. Also, the total number of registrations of common veterinary drugs (apart from vaccines) appears unrealistically low compared to other countries.

The number of PAS for which relevant products were found in the three lists ("List of Veterinary Drugs", "List of Veterinary Vaccine and Drugs Approved by CDSCO from 2010 to 2019" and "Veterinary Drugs & Vaccines Approved by CDSCO from January 2020 to September 2022") is very limited: only products containing nitroscanate (dogs only), suramin and buparvaquone appear to be registered (indicated with an asterisk (*) in Table 6).

In parallel to the three lists, a database Drugs@CDSCO can be found on the website: https://cdscoonline.gov.in/CDSCO/Drugs. However, the information in this database is a combination of animal and human drugs and for many registrations the target species is not clear. All substances for which one (or multiple) products were found in the database are indicated in Table 6. It cannot be excluded, however, that the registration actually concerns a product for human medicine.

The initial list ("List of Veterinary Drugs"), however, also contains some PAS not authorised in the EU that are lacking from the Indian MRL list. This concerns substances like furaltadone, furazolidone and nitrofurazone (nitrofuran drugs, EU prohibited), dimetridazole, carbadox, parbendazole, isometamidium, clopidol, and norfloxacin. Additionally, a registration for levofloxacin is present in the 2020-2022 list. Target species for particular veterinary drug registrations are often not indicated, but furaltadone, clopidol, dimetridazole, norfloxacin and levofloxacin registrations were found to apply to poultry.

Table 6 *Products found in India with substances not authorised in the EU (Drugs@CDSCO).*

	Substance	CDSCO database Registration	Target species	Approval year
A1c	Nandrolone	Y	Animal or human drug unclear	1962
A1c	Proligestone	N	-	-
A3b	Haloxon	N	-	-
A3b	1,2-Dichlorobenzene	N	-	-
A3b	Niclosamide	Y	Animal or human drug unclear	1965
A3b	Diethylcarbamazine	N	-	-
A3b	Nitroscanate*	(Y)	Dog	1996
A3b	Quinapyramine	Y	Animal or human drug unclear	1980
A3b	Suramin	Y*	Animal or human drug unclear	Approved in 1977
	Flavomycin[#]	Y	Used as feed additive in farm animals and poultry farm, birds	1989
A3d	Ethopabate	N	-	-
A3d	Dinitolmide (Zoalene)	Y	Animal or human drug unclear	1975 (Combination with ethoxyquin)
A3d	Buparvaquone*	Y	Bovine	1991
A3e	Gonadotropin	N	-	-
A3f	Acepromazine	N	-	-
A3f	Pentobarbitone	N	-	-
A3f	Promazine hydrochloride	N	-	-
A3f	Chloral hydrate	N	-	-
A3f	Lithium antimony thiomalate	N	-	-
A3f	Nimesulide	Y	Multiple products, primarily for humans. One product indicates: "For the treatment of pyrexia and inflammatory conditions in livestock".	Multiple years; product for livestock: 2004
A3f	Propofol	Y	Multiple products, some for humans, others unclear. One product indicates: "For induction in veterinary practise only".	Multiple years; product for veterinary practices: 2003
B1d	Antipyrine	N	-	-
-	Kaolin	Y	Animal or human drug unclear	1977 (Combinations with colistin, metronidazole or furazolidone)
-	Carboprost tromethamine	Y	Two products; one indicates: "…in cattle, mares and sheep.	1979;1986
-	Chloropyridazine	Y	Animal or human drug unclear	1990 (Cosumix plus)
-	Dexcloprostenolum	N	-	-
-	Mepyramine	N	-	-
-	Methyl hydroxybenzoate	Y	For livestock shrimp and fish	2009

[*] Product also found in the "List of Veterinary Drugs".

[#] Flavomycin (bambermycin) was banned as antimicrobial growth promoter in the EU, but recently reintroduced as veterinary drug for rabbits (no MRL necessary) and poultry (MRLs apply).

The evaluation of authorised VPPs in India proved to be difficult due to inconsistencies found in the available lists and databases. Furthermore, the available information is far from complete. As a result, it appears almost impossible to define underpinned analytical priority targets for residue monitoring in animal by-products originating from India.

3.5 Indonesia

The previous evaluation (van Asselt et al., 2021) showed that Indonesia had the highest number of authorised substances not included in EU legislation (n=44). However, the legislation on MRLs in Indonesia is very rudimentary. Matrix differentiation is limited to meat, eggs and milk, and it has not been updated over the last 20 years.

According to decree 52/KEPMEN-KP/2014 issued by the ministry of Marine Affairs and Fisheries (MMAF), several of the MRL substances indicated in Table 1 have been banned, at least for usage in aquaculture (Keputusan Menteri Kelautan dan Perikanan, 2014). These comprise: chloramphenicol, dapson, furazolidone, dimetridazole, ronidazole, trichlorfon and dichlorvos. Decree 52/KEPMEN-KP/2014 also mentions the substances explicitly approved for aquaculture, which appears a remarkably limited number of substances: tetracyclines, erythromycin and enrofloxacin. On the other hand florfenicol, one of the few antibiotics with explicit aquaculture registrations in Europe, is prohibited in Indonesia. Another interesting observation is the fact that many dyes are permitted drugs for aquaculture, although malachite green and crystal violet are prohibited substances (also in Europe). Also some anthelmintics (pyrantel, levamisole, praziquantel) for which usage in the EU is limited to land animals, are apparently used in aquaculture in Indonesia (Keputusan Menteri Kelautan dan Perikanan, 2014).

Registered VPP were retrieved from the online version of the Indeks Obat Hewan Indonesia. The site was developed by PT Gallus Indonesia Utama (GITA) which is a private organisation involved in publishing and consulting activities in the area of animal husbandry and health (https://gita-asohi.com/asohi/). Equivalent governmental information sources do not seem to exist. The database showed that out of the 44 substances initially found with an MRL in India (van Asselt et al, 2021), only 6 were represented in the product database (Table 7).

Table 7 *Products found in Indonesia with substances not authorised in the EU (Indeks Obat Hewan Indonesia).*

Substance	Nr of products	Target species	Remark
A1c - Testosteron	1	cattle, horse, goat, sheep, dog, cat, rabbit, turkey, goose, chicken, duck, bird	
A3b - Isometamidium	1	cattle, buffalo, camels, horses, donkeys and dogs	
A3b - Trichlorfon (metrifonate)	1	cattle, pigs, horses, sheep, goats, chickens, fish and larvae	According to MMAF decree prohibited substance
A3c - Kitasamycin	2	poultry, pigs, sheep, goats, cattle	
A3c - Clindamycin	2	not defined	
A3c - Norfloxacin	21	poultry, pigs, sheep, goats, cattle	

The only substance for which a substantial number of products is available is norfloxacin, a fluoroquinolone for which veterinary use is quite common outside Europe. Isometamidium is a drug used for treatment of trypanosomosis, a protazoal infection. Remarkably, even though the Decree 52/KEPMEN-KP/2014 indicates trichlorfon is prohibited, the veterinary products database returned a product based on trichlorfon. The number of products based on kitasamycin is modest compared to what was found in China, but applies to a larger group of target animal species.

Similar to what was observed for Brazil and India, the veterinary product database also appeared to contain a significant number of products based on substances lacking an MRL in Indonesia. These are displayed in Table 8.

Table 8 *Veterinary Pharmaceutical Products authorised in Indonesia containing additional substances that are not authorised for food producing species in the EU (Indeks Obat Hewan Indonesia).*

	Substance	Target species	No. of products	Remark
A1e	Ractopamine	Beef cattle, pig	2	-
A3b	Niclosamide	ruminants, chicken, duck, sheep, goat	17	-
-	Carbaryl	poultry, cattle, horse, sheep, pigs, dogs and cats	1	-
A3d	Diaveridine	poultry, turkey, rabbit	8	Combination sulfonamide
A3d	Pyrimethamine	poultry	8	Combination sulfonamide
A3b	Quinapyramine	cattle, horses, buffalo, camels, donkeys, elephants, goats/sheep, pigs, dogs	2	-
A3b	Dichlorfenthion	cattle, pigs, goats, sheep, dogs, chickens, fighting cocks and horses	1	-
A3b	Tetramisole	cattle, goats, sheep, pigs, horse, dogs	1	=levamisole + dexamisole
(A3c)	Ciprofloxacin	poultry, ruminants, pig	27	-
A3c	Cefuroxime	cows	1	-
A3c	Fosfomycin	poultry	9	-
A3c	Hexamine	poultry	2	-
A3c	Levofloxacin	poultry, sheep, goat	4	-
A3c	Ofloxacin	poultry	2	-
A3c	Pefloxacin	poultry	1	-
A3f	Phenylbutazone	poultry, horse, calves, cattle, goats, sheep, pigs, dogs and cats	1	-
A3c	Nystatin	poultry and swine	1	-

The list in Table 8 comprises several antibiotics not authorised for use in food producing animal species in the EU (ciprofloxacin, cefuroxime, fosfomycin, levofloxacin, ofloxacin, pefloxacin) and several other substances with an antimicrobial action (diaveridine, pyrimethamine, hexamine, nystatin). Quinapyramine is a trypanocidal drug like isometamidium. Niclosamide and tetramisole act as anthelmintics, while carbaryl and dichlorfenthion are insecticides.

In conclusion, only 6 substances out of the 44 identified in the initial survey yielded veterinary products, whereas authorised products were found based on a considerable number of additional substances not authorized in the EU. This indicates that also for Indonesia the available information is far from comprehensive.

4 Discussion

This study showed that it is a challenging task to obtain a comprehensive view on authorised veterinary drugs in countries outside the EU. Each country has its own legislation, registration process and administrative systems. For China and the USA, the obtained information seems consistent and complete. However, for Brazil, India and Indonesia, (major) inconsistencies were found. For India and Indonesia, the databases of registered VPPs seem to contain an unrealistically small number of products, also for EU-approved substances. This implies that the number of VPPs indicated in this report is likely to be underestimated. It should be noted that for Indonesia no official governmental database is available; the explored database is of private origin.

For Brazil, India and Indonesia, inconsistencies were found when comparing the content of VPP databases to local MRL regulations. For each of these countries, the search in VPP databases yielded a significant number of additional substances for which veterinary products appeared available, while no MRL is defined in national legislation. The inconsistencies found for India were supported by the latest HFAA audit https://ec.europa.eu/food/audits-analysis/audit-report/details/4055 (European Commission, 2018b):

- *"many pharmacologically active substances which are banned for use in food-producing animals in the EU are freely available on the Indian market and may be purchased over the counter".*
- *"The use of veterinary medicinal products in aquaculture is currently banned, so data on consumption do not exist".*
- *"Nitrofurans and chloramphenicol, banned in the EU for use in food producing animals, are not included in Schedule H, so they can legally be sold over-the-counter".*

Nevertheless, with respect to Brazil, China and the USA, the results of this study provide a more realistic view on PAS usage compared to the earlier report (van Asselt et al., 2021). Pharmaceutically active substances for which no products were found can be considered low priority for residue monitoring in animal by-products originating from these countries. This conclusion cannot be extended to India and Indonesia because the available databases were deemed incomplete.

Regarding the PAS for which VPPs were found, it remains difficult to determine the actual use, since this requires either consumption or sales data. However, the fact that they are on the market is an obvious indication that they may be used. When national legislation approves the use in livestock for domestic production, there is an obvious risk on presence of residues in animal by-products that are imported from these countries. It should be noted that the number of VPPs found for a specific PAS may not necessarily coincide with its actual use. Pharmaceutically active substances with a limited number of products may have a high use volume and vice versa.

It cannot be excluded that for some PAS, in particular substances belonging to the antiparasitics (group A3b), products are available that are not categorized as veterinary drug in a particular country, but rather as a biocide. In the EU, products can only be registered as veterinary medicinal product if the pharmaceutically active substance has been evaluated with the aim of establishing maximum residue limits, congregated in Regulation (EU) 37/2010. If a product is categorized as a biocide, pesticide MRLs apply. A few of the substances represented in Table 1 have pesticide MRLs for animal products in the EU: carbofuran, fenthion, fluvalinate, lindane, malathion and trichlorfon are represented in the EU pesticides database with MRLs between 1 and 100 ppb. It was beyond the scope of the current project to retrieve information on potential approved biocidal products available in the five countries of interest, nor did we pursue to identify whether or not in these countries a similar regulatory distinction is applied as in the EU.

Before initiating monitoring activities, an important aspect that needs to be considered with respect to enforcement, is the fact that no regulatory limits have been defined for PAS in animal by-products. In this study, many substances have been identified that could potentially occur in animal by-products. Past incidents have shown that these kind of findings inevitably raise questions with respect to acceptable

tolerance levels. Simply applying a zero-tolerance is debatable, merely because MRLs have been defined for animal products for human consumption, suggesting a safe level was established. Even for PAS formally prohibited in the EU, a legal basis for enforcement appears to be lacking, since it does not concern products for human consumption. Besides its relevance for enforcement, defining acceptable tolerance levels is also a prerequisite for method development; time and effort required for developing an analysis method will depend on the Limit of Detection (LoD) or Limit of Quantification (LoQ) requirements.

5 Conclusions and recommendations

5.1 Conclusions

Overall, in the current selection of five non-EU countries relevant as an origin of animal by-products, many veterinary pharmaceutical products (VPPs) containing pharmaceutically active substances (PAS) not authorised in the EU were found. Residue monitoring of imported animal (by-)products should preferably include these substances.

Considerable differences with regard to comprehensiveness of regulation was observed between countries. For the USA and China, VPPs were found for most of the substances for which maximum residue limits (MRLs) were identified in the prior study. In contrast, for these two countries only a minor number of products was found based on substances not identified before.

In contrast, for India and Indonesia hardly any registered products were found for the PAS initially deemed of interest. However, for these two countries as well as for Brazil, a considerable number of additional substances was found to be relevant, as VPPs for food producing species based on these substances were found.

Combining all information collected on availability of VPPs yields the overview of relevant PAS in Table 9.

Table 9 *Substances for which veterinary pharmaceutical products for food producing species were found.*

Substance	Category[1]	Brazil	China	United States	India	Indonesia	Relevant animal category
Azamethiphos	A3b	x					M
Berberine	-		x				M
Buparvaquone	A3d				*x*		
Carbadox	A3c			x	*x*		M
Carbaryl	-	x				x	M, P
Carboprost tromethamine	-				*x*		
Cefuroxim	A3c					x	
Chlorfenvinphos	A3b	x					
Chlorpromazine	A2		x				
Chlorpyridazine	-				*x*		
Chlorpyrifos	A3b	x					M, P
Clindamycin	A3c					x	
Clopidol	A3d		x	x	*x*		P
Coumaphos	B1b	x		x			M, P
Diaveridine	A3d	x				x	M, P
Dichlofenthion	A3b	x				x	M
Dichlorvos	A3b	x		x			M
Dimetridazole	A2		x		*x*		M, P
Diminazine	A3b	x	x				M
Dinitolmide (Zoalene)	A3d		x	x	*x*		P
Disofenol	A3b	x					M
Efrotomycin	A3c			x			M
Enramycin	A3c	x					M, P
Ergomethrin	-	x					M
Estradiol	A1c	x	x	x			M
Ethion	A3b	x					M
Ethopabate	A3d		x	x			P
Famphur	A3b			x			M
Fenitrothion	A3b	x					M

Substance	Category[1]	Brazil	China	United States	India	Indonesia	Relevant animal category
Fenprostalene	-			x			M
Fenthion	A3b	x		x			M
Fipronil	A3b	x					M
Flavomycin[#]	(A3c)		x	x	*x*		M, P
Fluvalinate	A3b		x				
Fosfomycin	A3c	x				x	M, P
Furaltadone	A2				*x*		
Furazolidone	A2				*x*		
Gentian violet	-	x					M
Gonadotropin	A3e		x	x			M, A
Haloxon	A3b			x			M
Halquinol	A3c	x					M, P
Hexamine	-					x	
Hygromycin B	A3c			x	*x*		
Isometamidium	A3b				*x*	x	M
Kaolin	-		x		*x*		M
Kitasamycin/leucomycin	A3c	x	x			x	M, P
Laidlomycin	A3d			x			M
Levofloxacin	A3c					x	
Lubabegron	A1e			x			M
Malathion	A3b		x				M, P
Melengestrol acetate	A1c	x		x			M
Mepyramine	-				*x*		
Metoserpate hydrochloride	A3f			x			P
Metronidazole	A2		x				M
Miconazol	A3c	x					M
Nandrolone	A1c		x		*x*		M
Niclosamide	A3b		x		*x*	x	M, A
Nimesulide	A3f				*x*		
Nitrofurazone	A2				*x*		
Nitroscanate	A3b				*x*		
Norfloxacin	A3c	x			*x*	x	M, P
Nystatin	A3c					x	
Ofloxacin	A3c					x	
Ormetoprim	A3d			x			P, A
Parbendazole	A3b				*x*		
Pefloxacin	A3c					x	
Phenylbutazone	A3f	x				x	M
Propetamphos	A3b		x				M
Propofol	A3f				*x*		
Propoxur	-	x					M, P
Pyrantel	B1b			x			M
Pyrimethamine	A3d					x	
Quinapyramine	A3b				*x*	x	
Ractopamine	A1e			x		x	M, P
Scopolamine	-		x				M
Sulfomyxin	A3c			x			
Suramin	A3b				*x*		
Testosterone	A1c	x	x	x		x	M
Tetramethrin	A3b	x					M, P
Tetramisole	A3b					x	
Thiabendazole	A3b			x			
Trenbolone	A1c			x			M
Trichlorfon	A3b	x	x			x	M, P, A
Tripelennamine	-			x			M
Zeranol	A1d			x			M
Zilpaterol	A1e			x			M

[1] Category according to Implementing Regulation (SANTE 10216-2022).

M: mammalian; P: poultry; A: aquaculture.

5.2 Recommendations

It is recommended to include the substances, for which veterinary products were found, in the monitoring program of imported animal by-products as part of the National Plan Animal Feed. The outcome of this research may also be used to support risk-based analysis of animal products such as meat, dairy in eggs for the National Residue Control Plan (NRCP), since residues may be expected in imports of these products as well.

However, before initiating monitoring, it is highly recommendable to define tolerance levels for PAS in animal by-products, in order to facilitate enforcement. Also a critical assessment of the exact nature (and volumes) of the imported by-products should be performed on individual country-level, before initiating actual monitoring. This enables prioritization of matrices with respect to method development. Since the actual risk of residues occurring in animal by-products also depends on the pharmacokinetics of the PAS, data on pharmacokinetics could also be taken into account for prioritization.

The establishing of tolerance levels and matrix prioritization serve as prerequisites for method development, which will be very challenging, considering the number and broad chemical spectrum of substances of interest. It is inevitable that choices will have to be made, since it is unrealistic to develop a fully comprehensive analysis method.

Future HFAA missions to Brazil, India and Indonesia should focus more thoroughly on the comprehensiveness of the regulation and registration of VPPs for food producing animal species since many inconsistencies were found for these countries. It should be acknowledged that availability of information on registered products may be inadequate, and it is obvious that when MRL legislation is taken as a starting point, availability of additional substances may be overlooked.

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References

EURL. (2022). EURL Guidance on Minimum Method Performance Requirements (MMPRs) for Specific Pharmacologically Active Substances in Specific Animal Matices. Retrieved 06-03-2023, from https://food.ec.europa.eu/system/files/2022-06/cs_vet-med-residues_guideline_eurl_mmpr.pdf.

European Commission. (2018a). Brazil 2018-6349. Retrieved 06-03-2023, from https://ec.europa.eu/food/audits-analysis/audit-report/details/4053.

European Commission. (2018b). India 2018-6345. Retrieved 06-03-2023, from https://ec.europa.eu/food/audits-analysis/audit-report/details/4055.

Keputusan Menteri Kelautan dan Perikanan. (2014). Keputusan Menteri Kelautan dan Perikanan tentang Klasifikasi Obat Ikan. Retrieved 06-03-2023, from https://peraturan.bpk.go.id/Home/Details/159953/kepmen-kkp-no-52kepmen-kp2014-tahun-2014.

van Asselt, E. D., Pikkemaat, M. G., Hobe, R. G., Meijer, N. P., van Egmond, H., & Bikker, P. (2021). *Potential occurrence of veterinary pharmaceuticals in animal by-products - An inventory of authorised substances in non-EU countries that are not allowed within the EU*.

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